

Ballast Water Management System
with PERACLEAN® Ocean “SKY-SYSTEM®”

Report of Additional Land-Based Test

Date Submitted: June 12, 2012

NIPPON YUKA KOGYO Co., Ltd.

KATAYAMA CHEMICAL, INC.

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Ballast Water Management System
with PERACLEAN® Ocean “SKY-SYSTEM®”

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Laboratory of Aquatic Science Consultant Co., Ltd.



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Statement

Title of test

Additional land-based tests for “Ballast Water Management System with PERACLEAN® Ocean (SKY-SYSTEM®)”

Test Number

T-40-140 (Additional Land-Based Test Plan)

With reference to Ballast Water Management System “Ballast Water Management System with PERACLEAN® Ocean (SKY-SYSTEM®) (hereafter SKY-SYSTEM®)” which has added a neutralization control unit when discharging ballast water, land-based tests under sea water and brackish water have been implemented in accordance with “Standard for type approval appraisal test on ballast water management system” (Inspection and Measurements Division, Maritime Bureau, Ministry of Land, Infrastructure, Transport and Tourism, Japan). In addition to the compliance evaluation of SKY-SYSTEM® with Regulation D-2 of the International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 (hereinafter referred to as “the BW convention”), this test also included developments of sample water and its transportation for aquatic toxicity tests and tests to confirm by-products generation of ballast water discharge.

In order to perform tests according to quality assurance with international standards, “quality management plan (QMP) and quality assurance project plan (QAPP) of the approved testing procedure (D-01-140) and additional land-based test plan (T-40-140)” were developed by Laboratory of Aquatic Science Consultant Co., Ltd. (hereafter the company), taken into consideration ISO/IEC17025 and quality management system (JISQ9001:2000 equivalent to ISO9001:2000) and applied to this test.

Responsible Party for the Test:

Laboratory of Aquatic Science Consultant Co., Ltd.

Kei Kushiro

June 12, 2012

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Credential of Quality Performance

Title of test

Additional land-based tests for “Ballast Water Management System with PERACLEAN® Ocean (SKY-SYSTEM®)”

Test number

T-40-140 (Additional land-based test plan)

Regarding the test, inspections or audits by the party responsible for reliability assurance work were implemented as per the table below, and the results thereof were reported to the responsible person for the test.

Date of audit	Items to be audited	Date of report
Sept.,10, 2011	Test plan	Sept.11, 2011
Sept.,30, 2011	Test facility/equipment-instrument, SOP etc.	Oct.1, 2011
Oct.25, 2011 and Jan.17, 2012	Implementation State of Test	Jan. 20, 2012
May 21, 2012	Report (draft)	May 25, 2012
June 1, 2012	Final report	June 12, 2012

The undersigned assures that the above-stated tests were implemented according to the Test Plan, and the raw data obtained in the tests concerned were accurately reflected in the statement of the Report.

Responsible Party for Reliability Assurance Work:
Laboratory of Aquatic Science Consultant Co., Ltd.

Ysuhiko Ohki
June 12, 2012

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1. Applicability

With reference to the SKY-SYSTEM® which has been installed a neutralization process during ballast water discharge, full-scale land-based tests (treatment flow rate: 200m³/h) were implemented in accordance with the standard for type approval appraisal test of ballast water management system (Inspection and Measurements Division, Maritime Bureau, Ministry of Land, Infrastructure, Transport and Tourism, Japan). In addition to the compliance evaluation with the Regulation D-2 (hereinafter referred to as “D-2”) of the BW convention, this test also included developments of test water and its transportation for aquatic toxicity tests and tests to confirm disinfection by-product generation of the ballast water discharge.

In order to perform tests according to quality management with international standards, “Quality Management Plan(QMP) and Quality Assurance Project Plan (QAPP) of the Approval Testing Process (D-01-130)” and Additional Land-Based test Plan (T-40-140) were developed by the Laboratory of Aquatic Science Consultant Co., Ltd. (hereafter the company), taken into consideration ISO/IEC17025 and quality management system (JIS Q9001:2000 (equivalent to ISO 9001: 2000) for the purpose of providing a consistent products and services to satisfy clients requirements and regulatory request, continuous improvement of system and effective applications and applied to this test.

2. Summary of test results

Test water was prepared to comply with “Ballast Water Management System Type Approval Test Standard for Land-based test” (hereinafter referred to as “standard for land-based test”) and total 4 tests constituting 1 test for test water with salinity > 32psu (hereinafter referred to as “sea water requirement”) and 3 tests for test water with salinity 3 - 32psu (hereinafter referred to as “brackish water requirement”) were implemented with methods to comply with the standard for land-based test.

Among 4 tests, test to evaluate compliance with the D-2 standard was 2 times, one for sea water requirement and another for brackish water requirement. After chemical preparation (PERACLEAN® Ocean) was injected to achieve prescribed concentrations (150 mg/L (22.5 mg/L as PAA and 21.0 mg/L as H₂O₂)) under the control of “SKY-SYSTEM®” and stored for 1 day in the water storage tank, it was treated by neutralization (injection of 1,887-1,079 mg/L sodium sulfite) when discharged. L size group, S size group and bacteria (*Escherichia coli*, *Enterococci* and toxic *Vibrio cholerae*

(serotype O-1 and O-139)) in this treated discharge water found all the concentrations were less than the D-2 standard specified for land-based test.

Further, PAA and H₂O₂ in the discharge water after neutralization were less than 0.3 mg/L and 0.5 mg/L respectively.

Test water for aquatic toxicity test was sampled at the same time or in the same method of compliance test with the D-2 standard as described above and was transported properly under the dark and cold conditions so that it could be disclosed to test organisms within 10 hours after sampling.

3. Time table of test

Time table of tests is shown in table 3-1.

Table 3-1 Time table of tests

	Date of ballasting (treatment)	Date of discharge	Reference
Sea water requirement: 1 st (Test run 22 (TR 22))	2011/10/25	2011/10/26	Compliance test with D-2 standard, development of test water and transportation for aquatic toxicity test and test to confirm by-product generation
Brackish water Requirement: 1 st (Test run 23 (TR 23))	2011/11/07	2011/11/08	Development of test water and transportation for test to confirm by-product generation
Brackish water Requirement : 2 nd (Test run 31 (TR 31))	2012/01/16	2012/01/17	Development of test water and transportation for compliance test with D-2 standard and test to confirm by-product generation
Brackish water Requirement: 3 rd (Test run 35 (TR 35))	2012/03/28	2012/03/29	Development of test water and transportation for aquatic toxicity test and test to confirm disinfection by-product generation

4. Test system

This test was implemented using a full-scale land-based test facility (treatment flow rate: 200m³/h) installed at a public quay of Imari city, Saga, Japan (Figure 4-1). This test facility was installed by Nippon Yuka Kogyo Co., Ltd. and Katayama Chemical, Inc. who are both developer of the SKY-SYSTEM®.

This test facility was primarily constituted of 2 units of raw water tank (200 m³) to store raw water prior to the treatment, a water quality conditioner tank to store and inject water quality preparations and test organisms, 2 units of sampling equipment to sample test water on ballasting and discharging, a flow meter, a ballast pump, a treated water tank to store treated water (200 m³), an untreated water tank (200 m³) to store untreated/control water, a storage tank and an injection unit of PERACLEAN® Ocean a storage tank and an injection unit of the neutralizer, storage tank and injection unit of sodium sulfite as neutralizer, PO (PERACLEAN® Ocean) concentration monitoring unit-1 and -2 to monitor concentrations, and a control panel to control injection concentration and injection amount of neutralizer.

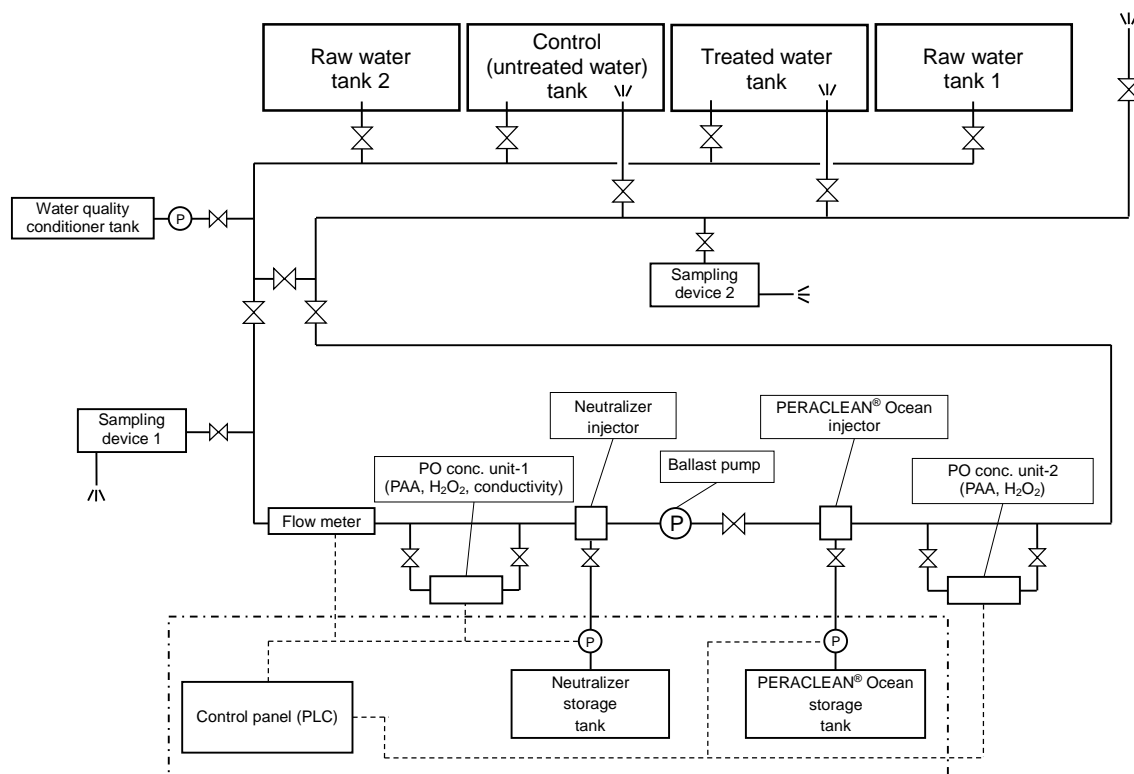


Figure 4-1 Schematic diagram of additional land-based tests facility

5. Test method

Methods applied to each test were as follows. The following items for all the tests were recorded to confirm the normal operation of the land-based test facility.

- i. Flow rate on ballast water line;
- ii. Injection amount and concentration of PERACLEAN® Ocean during ballasting treatment ;
- iii. Concentration of PAA and H₂O₂ by the PO concentration monitoring unit-1 and unit-2 when discharging treated water;
- iv. Injection amount and concentration of 15% aqueous solution of sodium sulfite as the neutralizer when the treated water was discharged and the concentration of PAA and H₂O₂ after injection by the PO concentration monitoring unit-2; and
- v. Flow rate to sampling equipment on sampling;
- vi. Whether or not dysfunction of system occurred and if so its detail.

5.1 Sea water requirement: 1st run (Test run (TR) 22)

This test was implemented to evaluate the compliance with the D-2 standard under the condition of the sea water requirement and also to develop (including transportation) test water for aquatic toxicity test and test to confirm generation of disinfection by-products.

On ballasting, raw water from the raw water storage tank flow continuously by ballast pumps (flow rate: 200m³/h) and ultimately treated water and untreated/control water were stored at the treated water tank and the untreated water tank, respectively (see Figure 5-1 and -2).

Tetraselmis sp. (Yanmar Co., Ltd.) as phytoplankton *Brachionus plicatilis* (Chlorella Industry Co., Ltd.) as zooplankton were injected from the water quality conditioner tank to both treated water and untreated/control water as test organisms in such manner to give 1.0 x 10³ cells/ml and 1.0 x 10⁵ inds./m³, respectively, under the concentration of flowing ballast water. Granulated sugar (Dai-nippon Meiji Sugar Co., Ltd.), cornstarch yellow (Japan Corn Starch Co., Ltd.) and Sylycia 310P (FUJI SYLYSIA CHEMICAL LTD.) were also injected from the water quality conditioner tank as water quality conditioning agents in such manner to keep the concentration of POC, DOC and

TSS no less than 1.0 mg/L in the flowing ballast water.

Operation on discharge began after 1 day (24 hour) storage from the ballasting. Treated water flow consequentially (flow rate: 200m³/h) from the treated water tank and the untreated water tank through the ballast pump and was ultimately discharged to the sea. In the case of treated water, dosing amount of the neutralizer which could completely neutralize PO required concentration (150 mg/L (22.5 mg/L as PAA and 21.0 mg/L as H₂O₂)) in response to the salinity was automatically injected from the neutralizer storage tank for a period of 7.5 minutes since the beginning of ballast water discharge. During that period, the response to concentrations measurement of Active Substances by the PO monitoring unit-1 and unit-2 were secured. Then the PO monitoring unit-1 measured concentrations of Active Substances (PAA and H₂O₂) and salinity and if such data as more than 0.3 mg/L of PAA and more than 0.5 mg/L of H₂O₂ were perceptible, necessary dosing amount of neutralizer is automatically injected from the neutralizer storage tank. Since the concentration of Active Substances after the neutralization process (PAA no more than 0.3mg/L and H₂O₂ no more than 0.5mg/L) is confirmed by the PO monitoring unit-2, discharge to the sea starts (see Figure 5-3). Untreated/control water is discharged from the untreated tank to the sea through the ballast pump. Various samplings were conducted just before discharge to the sea by dividing with “isokinetic flow speed” at the sampling equipment-2 (see Figure 5-4).

Analysis of various samplings related to compliance evaluation with the D-2 standard including measurement of water quality and analysis, took place at the public quay test site on Imari bay, Saga Prefecture, Japan in accordance with “Quality management plan (QMP) and quality assurance project plan (QAPP) of the approval testing process (D-01-030)” and the additional land-based test plan (T-40-140). Analysis of test water on both ballasting and discharge started within 6 hours after sampling.

Test water for an aquatic toxicity test and a test to confirm disinfection by-product generation (raw water prior to treatment, immediately after treatment, discharge water after neutralization) was transported under the dark and cool conditions to Idea Consultants, Inc., Yaizu City, Shizuoka Prefecture, Japan within 10 hours after completion of discharge. Test water on ballasting (raw water prior to treatment, immediately after treatment) was stored for 24 hours until completion of discharge under the dark and cool conditions at the coastal test site on Imari bay, Saga Prefecture, Japan.

5.2 Brackish water requirement: 1st run (TR 23)

This test was implemented to develop (and transport) test water for disinfection by-product generation confirmation test under the brackish water requirement.

Sampling was carried out prior to treatment, prior to neutralization after 1 day storage and after neutralization.

Test raw water was previously diluted with spring water and its salinity was adjusted to brackish water requirements applicable to the land-based test standard.

Operations during ballasting was to flow (flow rate: 200m³/hr) continuously the raw water from the raw water storage tank through the ballast pump, inject PERACLEAN® Ocean (150 mg/L) to the flow, prepare test water prior to treatment by dividing the flow with “isokinetic flow speed” through sampling equipment-1 and store at the treated water tank (see Figure 5-1).

Tetraselmis sp. of phytoplankton (Yanmar Co., Ltd.) and *Brachionus plicatilis* of zooplankton (Chlorella Industry Co., Ltd.) were injected from the adjustment tank as test organisms to the source water prior to treatment on ballasting in such manner as to give concentrations of 1.0 x 10³ cells and 1.0 x 10⁵ ind./m³, respectively. Granulated sugar (Dai-nippon Meiji Sugar Co., Ltd.), cornstarch yellow (Japan Corn Starch Co., Ltd.) and Sylysia 310P (Fuji Silysia Chemical Ltd.) were added from the water quality conditioner tank as water quality conditioning agents in such manner to achieve more than 5 mg/L for POC, more than 5 mg/L for DOC and more than 50 mg/L for TSS in the flowing ballast water.

Treated water after 1 day storage flew (flow rate: 200m³/hr) continuously from the treated water tank through the ballast pump and a neutralizer dose amount which could neutralize completely the particular PO concentration (150 mg/L (22.5mg/L as PAA and 21.0mg/L as H₂O₂)) responding to the salinity measured was automatically injected from the neutralizer storage tank for a period of 7.5 minutes since the beginning of ballast water discharge. During that period response to the concentration measurement of the Active Substances by the PO concentration monitoring units secured. Then measurement of concentration of the Active Substances (PAA and H₂O₂) and salinity by the PO concentration monitoring unit-1, automatic injection of proper neutralizer dose based on the measurement data, monitor of PAA and H₂O₂ (PAA: less

than 9.3 mg/L, H₂O₂: less than 0.5 mg/L) by the PO monitoring unit-2, sampling of test water after neutralization by dividing with “isokinetic flow speed” through sampling equipment-2 and discharge to the sea follow (see Figure 5-3).

Water temperature, pH, salinity, dissolved oxygen concentration, and turbidity of sampled test water were immediately measured by instruments. After samples for TSS, POC, DOC analysis had been developed, they were transported to Idea Consultants, Inc., Yaizu City, Shizuoka Prefecture, Japan under the dark cold condition in 10 hours after sampling.

5.3 Brackish water requirement: 2nd run (TR 31)

This test was performed to develop (also transport) test water for compliance test with D-2 standard as well as test to confirm by-product generation.

Salinity adjustment and test methods during ballasting and discharge are basically same as “5.1 Sea water requirement: 1st run (TR 22)”. Addition of test organisms and water quality adjuster is same as “5.2 Brackish water requirement: 1st run (TR 23)”.

5.4 Brackish water: 3rd run (TR 35)

This test was performed to develop (also transport) test water for aquatic toxicity tests as well as tests to confirm by-product generation under the brackish water requirement.

Natural sea water to develop test raw water was diluted with public water which had been previously dechlorinated and salinity of test raw water met brackish water requirement for the land-based test standard. Test methods during ballasting and discharge are basically same as “5.1 Sea water requirement: 1st run (TR 22)”. Addition of Test organisms and water quality adjuster is same as “5.2 Brackish water requirement: 1st run (TR 23)”.

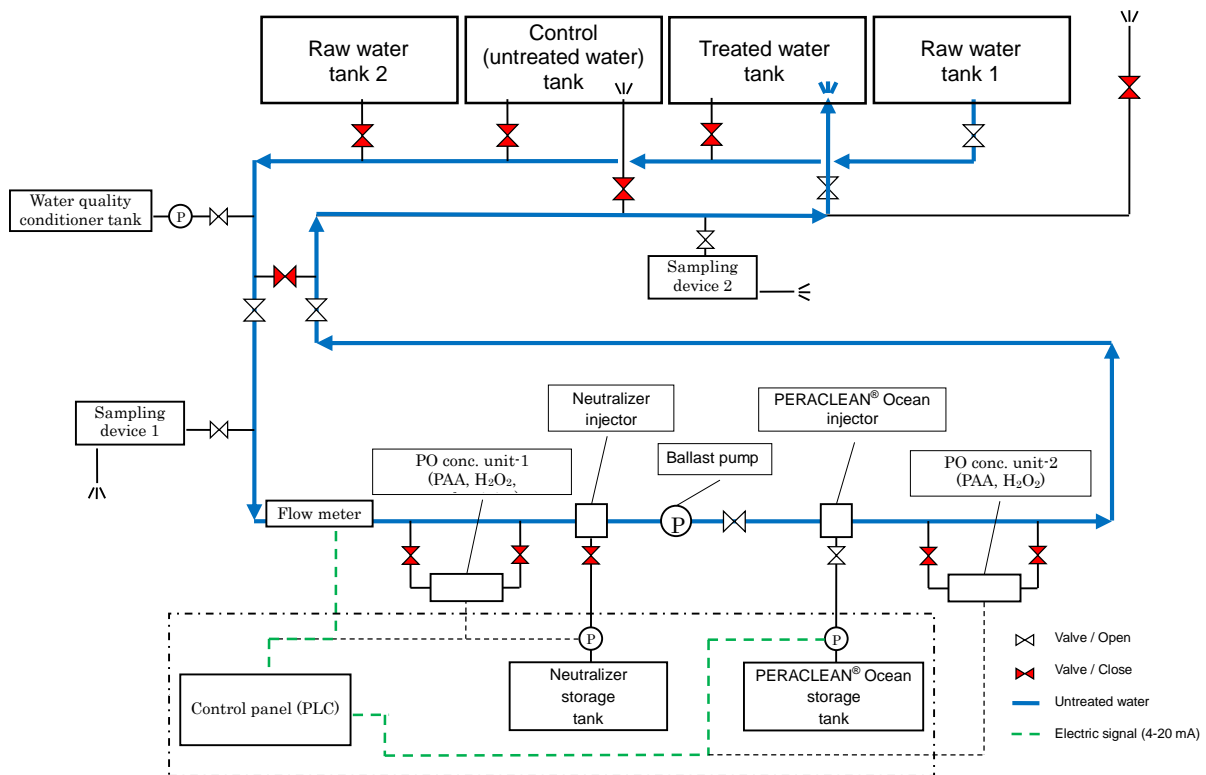


Figure 5-1 Flow of treated water during ballasting

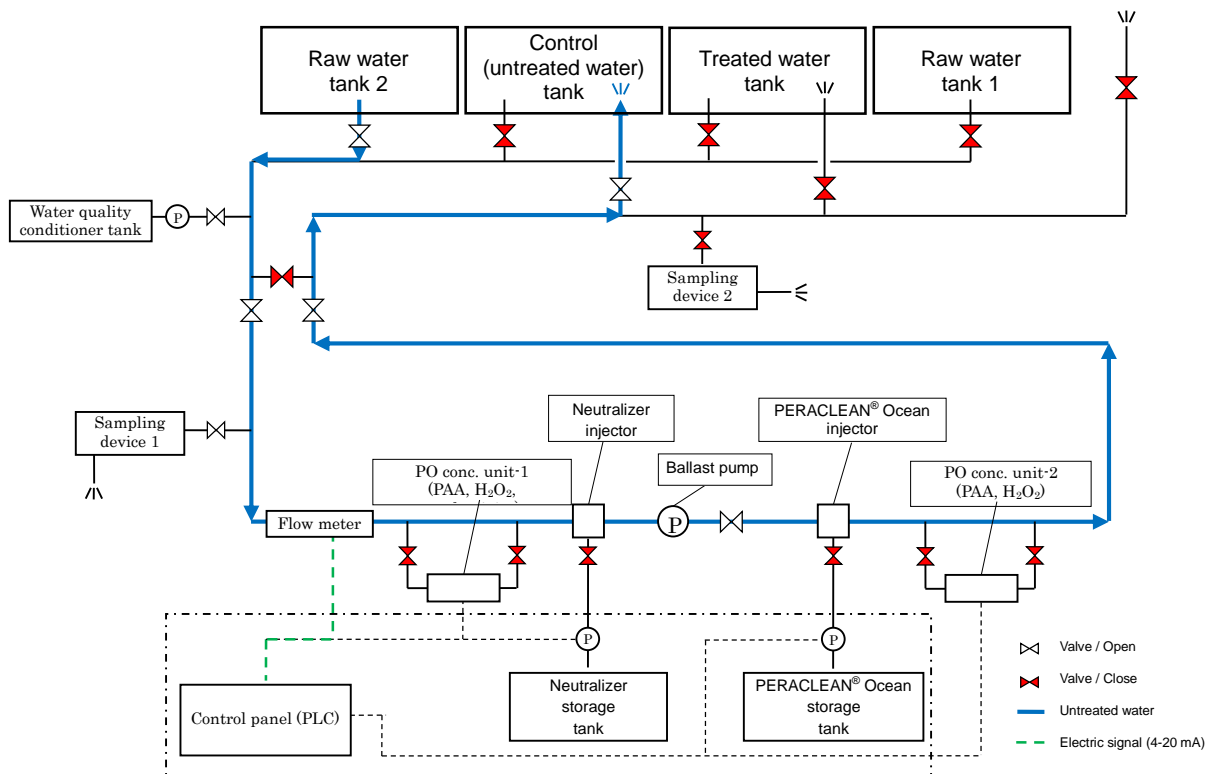


Figure 5-2 Flow of untreated/control water during ballasting

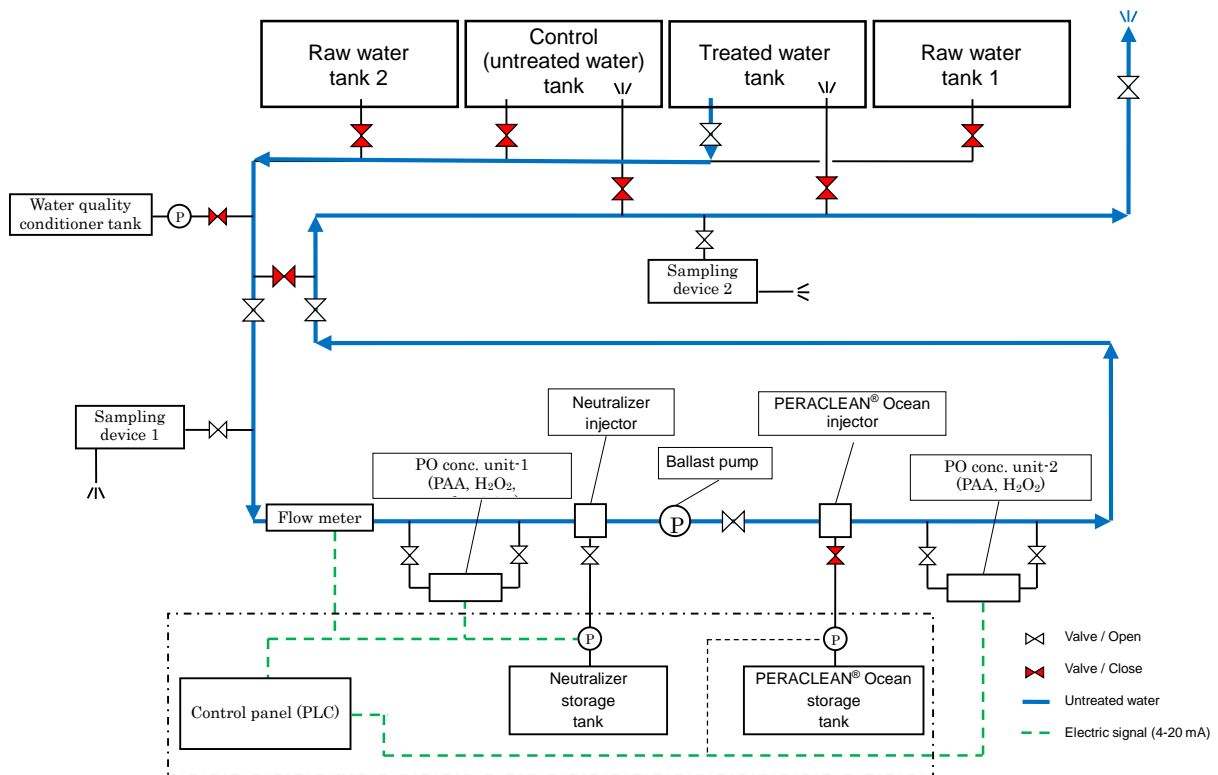


Figure 5-3 Flow of treated water during discharge

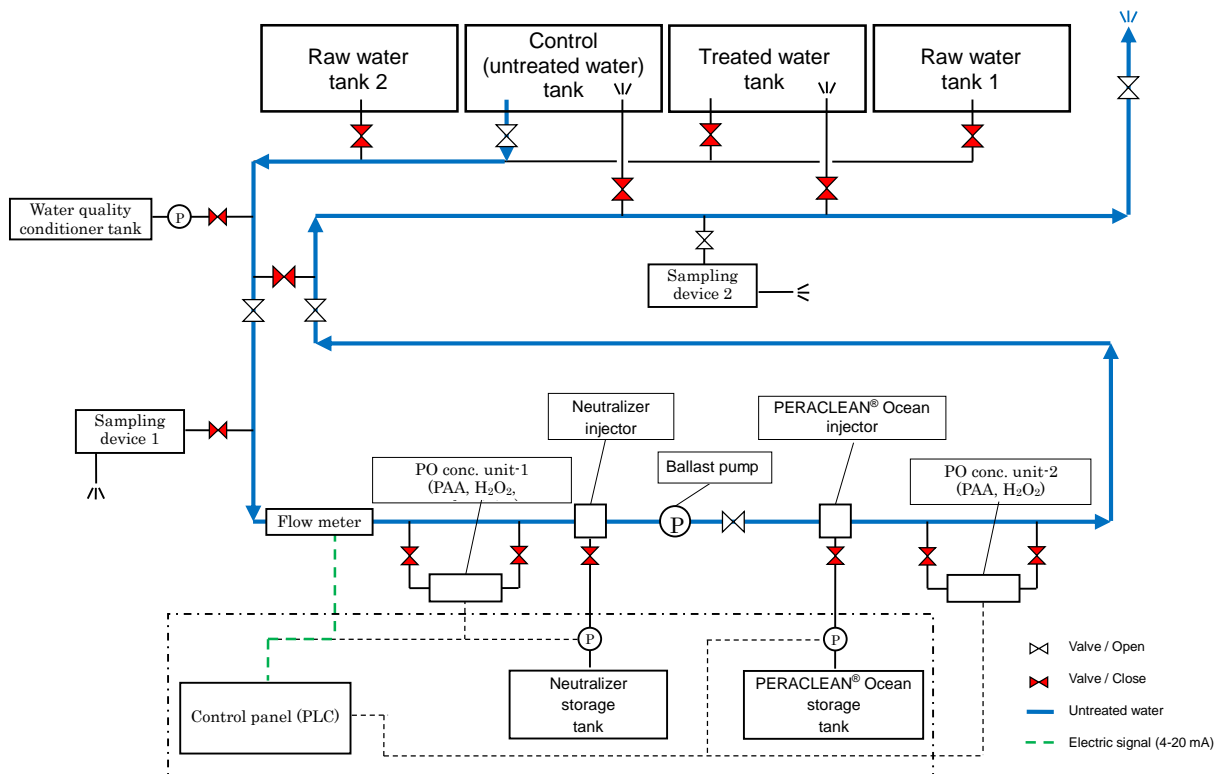


Figure 5-4 Flow of untreated/control water during discharge

6. Test result

6.1 Sea water requirement: 1st run (TR 22)

(1) Evaluation on compliance with the D-2 standard

1) L size group

Table 6.1-1 shows test result for L size group.

Since untreated/control water during ballasting and discharge showed more than 10^5 inds./m³ and 10^2 inds./m³, respectively, the tests were qualified as eligible one. Also since treated water discharge showed less than 10^1 inds./m³, the D-2 standard was accomplished.

Table 6.1-1 Test result of L size group with sea water requirement (1st run (TR 22))
(unit: individual number (inds./m³))

		The total number of L size	The number of taxonomic groups	The number of species
Control	Beginning	1.2 ×10 ⁵	3	10
	Middle	1.1 ×10 ⁵	3	10
	End	1.0 ×10 ⁵	3	10
Before treatment	Beginning	1.3 ×10 ⁵	3	13
	Middle	1.4 ×10 ⁵	4	12
	End	1.2 ×10 ⁵	3	11
Immediately after treatment	Beginning	3 ×10 ⁰	1	2
	Middle	1 ×10 ⁰	1	1
	End	N.D.	N.D.	
One day after control	Beginning	5.4 ×10 ²	4	16
	Middle	4.6 ×10 ²	4	11
	End	4.1 ×10 ²	3	14
One day after treatment	Beginning	N.D.	N.D.	
	Middle	N.D.	N.D.	
	End	N.D.	N.D.	

N.D. indicates that organisms were not detected.

2) S size group

Table 6.1-2 shows test results of S size group.

Since untreated/control water showed more than 10^3 inds./ml during ballasting and more than 10^2 inds./ml during discharge, the tests were qualified as eligible one. Also treated water discharge showed less than 10^1 inds/ml and accomplished the D-2 standard.

Table 6.1-2 Test result of S size group with sea water requirement (1st run (TR 22))

(unit: individual number (inds./ml))

		The total number of S size	The number of taxonomic groups	The number of species
Control	Beginning	2.49 $\times 10^3$	4	12
	Middle	2.60 $\times 10^3$	4	15
	End	2.81 $\times 10^3$	4	11
Before treatment	Beginning	2.59 $\times 10^3$	3	14
	Middle	2.43 $\times 10^3$	4	16
	End	2.63 $\times 10^3$	4	15
Immediately after treatment	Beginning	0.55 $\times 10^0$	1	3
	Middle	0.27 $\times 10^0$	1	2
	End	0.27 $\times 10^0$	1	1
One day after control	Beginning	15.90 $\times 10^2$	4	10
	Middle	15.20 $\times 10^2$	4	10
	End	16.76 $\times 10^2$	4	10
One day after treatment	Beginning	N.D.	N.D.	N.D.
	Middle	0.06 $\times 10^0$	1	3
	End	0.01 $\times 10^0$	1	1

N.D. indicates that organisms were not detected.

3) Bacteria

Table 6.1-3 shows test result of bacteria.

The land-based test standards require test water containing at least 10^4 cfu/ml of heterotrophic bacteria. This test satisfied the requirement.

(1) *Escherichia coli*

Colony forming unit number of *Escherichia coli* in the treated water on discharge (after 1 day storage) was lower than standard of the ballast water discharge (less than 250 cfu/100 ml).

(2) Enterococci

Colony forming unit number of Enterococci in the treated water on discharge (after 1 day storage) was lower than standard of the ballast water discharge (less than 100 cfu/100 ml).

(3) Toxicogenic *Vibrio cholerae*

Colony forming unit number of toxicogenic *Vibrio cholerae* (*Vibrio cholera* O-1 and O-139) in the treated water on discharge (1 day storage) was lower than standard of ballast water discharge (less than 1 cfu/100 ml).

Table 6.1-3 Test result of Bacteria under sea water requirement (1st run (TR 22))

	Control						Treatment								
	Ballasting			One day after			Before			Immediately			One day after		
	Beginning	Middle	End	Beginning	Middle	End	Beginning	Middle	End	Beginning	Middle	End	Beginning	Middle	End
Toxicogenic <i>Vibrio cholerae</i> (O1,O139)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Escherichia coli</i>	5,600	5,000	3,400	120	120	80	1,000	4,000	2,200	N.D.	N.D.	N.D.	N.D.	0.40	0.20
Enterococci	12	3.1	0.79	N.D.	N.D.	N.D.	N.D.	0.43	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.15
Heterotrophic bacteria	91,600	68,000	78,600	668,000	600,000	550,000	102,600	92,400	77,400	24	24	30	8,500	9,400	11,560

Unit: Toxicogenic *Vibrio cholerae*, *Escherichia coli* and Enterococci (cfu/100 ml), Heterotrophic bacteria (cfu/ml)

N.D. means that aforementioned bacteria were not detected.

4) Water quality

Table 6.1-4 shows results of water quality (water temperature, pH, salinity, dissolved oxygen, turbidity, TSS, POC, DOC).

Land-based test standard specifies salinity, TSS, DOC, POC in the source water prior to treatment and untreated/control water on ballasting.

More than 32psu for salinity, more than 1mg/L for TSS , more than 1mg/L for POC, and more than 1mg/L for DOC are required for sea water requirement.

This test has achieved these standards of water quality.

Table 6.1-4 (1) Result of measurement and analysis of water quality for sea water requirement
(1st run (TR 22): treated water)

Process	sampling	Temp (°C)	pH value	Salinity (PSU)	DO (mg/L)	Turbidity (NTU)	TSS (mg/L)	POC (mg/L)	DOC (mg/L)
in take: before treatment	beginning	19.5	8.1	32.3	7.7	20.6	14	3.5	2.4
	middle	19.8	8.1	32.2	7.3	27.8	13	3.6	2.5
	end	20.1	8.2	32.2	7.3	28.2	13	3.5	2.2
intake: immediately after treatment	beginning	19.5	6.7	32.3	10.1	32.6	12	3.2	22.1
	middle	19.6	6.7	32.1	9.3	30.3	13	3.1	24.6
	end	20.0	6.7	32.2	8.4	28.2	13	2.5	23.3
discharge: before neutralization	beginning	19.9	6.6	32.2	10.9	7.9	6	2.1	18.8
	middle	19.8	6.6	32.2	11.4	7.0	5	1.9	18.9
	end	19.8	6.6	32.2	11.8	8.3	5	1.9	18.8
discharge: after neutralization	beginning	19.9	6.9	32.1	2.3	6.9	4	1.4	21.5
	middle	20.2	7.0	32.2	1.4	13.1	5	1.5	21.6
	end	20.1	6.9	32.1	1.2	13.9	4	1.5	20.5

Table 6.1-4 (2) Result of measurement and analysis of water quality for sea water requirement
(1st run (TR 22): untreated/control water)

Process	sampling	Temp (°C)	pH value	Salinity (PSU)	DO (mg/L)	Turbidity (NTU)	TSS (mg/L)	POC (mg/L)	DOC (mg/L)
in take: control	beginning	20.0	8.0	33.9	7.5	21.7	12	3.6	2.4
	middle	20.1	8.0	34.0	7.4	31.1	14	3.5	2.3
	end	20.4	8.0	33.9	7.1	36.2	13	3.6	2.4
discharge: control	beginning	20.1	8.0	33.8	7.7	7.7	5	1.2	2.5
	middle	20.3	8.0	33.9	7.1	9.6	6	1.4	2.6
	end	19.9	8.0	33.9	7.3	10.0	6	1.2	2.5

5) Others (items to be recorded in tests)

The following items were recorded in tests:

- .1 Flow rate on ballast line;
- .2 Dosing amount and concentration of PERACLEAN® Ocean in treatment during ballasting;
- .3 Concentration of active substances (PAA and H₂O₂) by the PO concentration monitoring unit-1 and unit-2 when discharging treated water;
- .4 Dosing amount and concentration of sodium sulfite as a neutralizer when discharging treated water and concentration of PAA and H₂O₂ by the PO concentration monitoring unit-2 when (after) adding;
- .5 Amount flowing to sampling equipment when sampling; and
- .6 Whether or not a dysfunction of system occurs and if so detail of accident.

Figure 6.1-1~3 show log data of these recording items of this test.

Flow amount was stable in the neighborhood of a little more than 200m³/h. Dosing amount and concentration of PERACLEAN® Ocean during treatment when ballasting were 27 L/h as projected (in terms of ballast water amount of 207 m³/h) and 150 mg/L. without much fluctuation.

Concentration of PAA and H₂O₂ before neutralization during discharge of treated water was about 1.2 mg/L and 11.2 mg/L respectively and injection of sodium sulfite as a neutralizer lowered them under detectable limit.

No particular obstacle in recording and operation has not been observed. This means this test system has been controlled as planned.

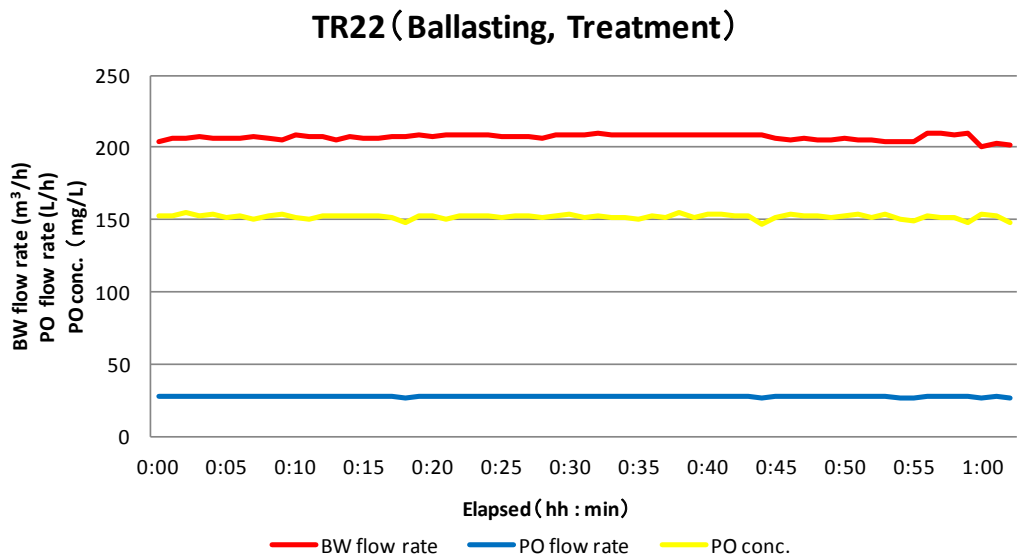


Figure 6.1-1 Control of treated water during ballasting

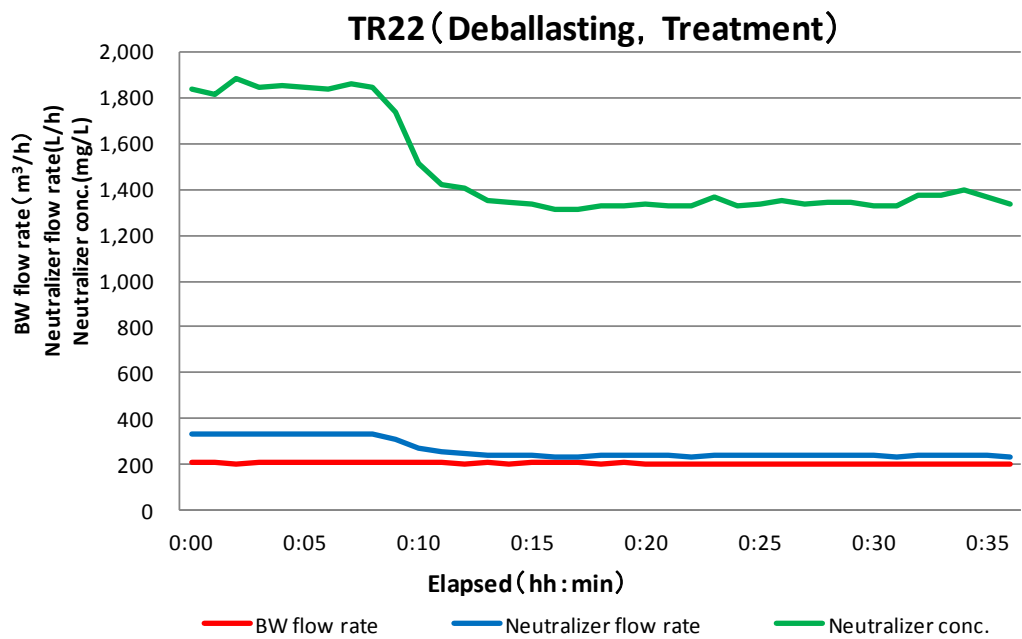


Figure 6.1-2 Control of treated water during discharge (1)

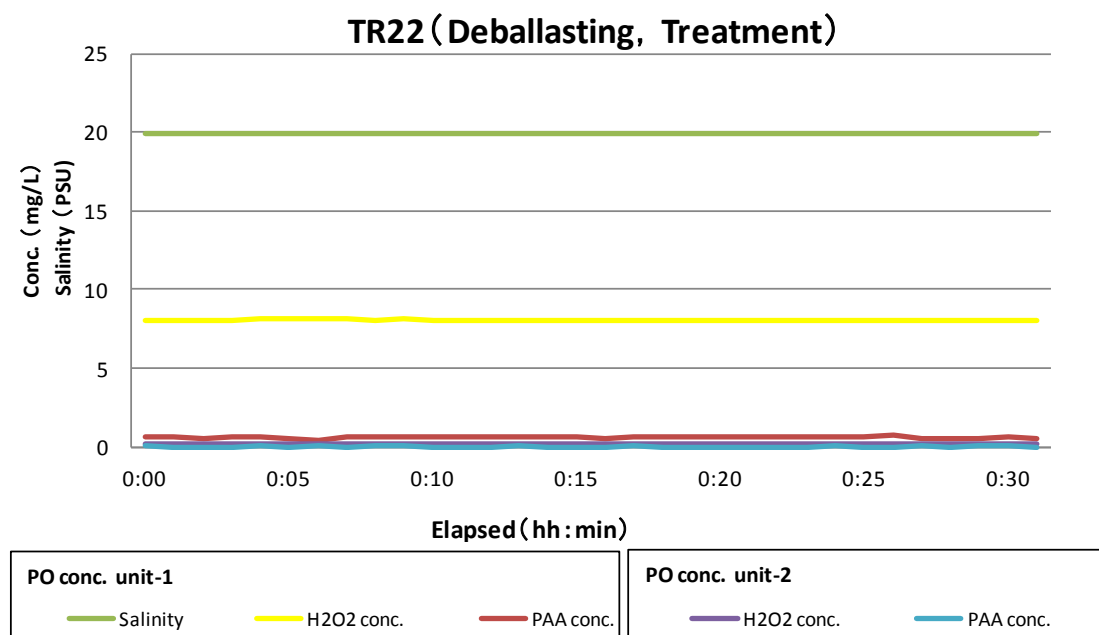


Figure 6.1-3 Control of treated water during discharge (2)

(2) Development and transportation of test water for aquatic toxicity test and test to confirm by-product generation

Test water for aquatic toxicity test and test to confirm by-product generation (source water prior to treatment, immediately after treatment and discharge water after neutralization) was transported to Idea Consultants, Inc., Yaizu City, Shizuoka Prefecture, Japan under the dark cold condition in 10 hours after completion of discharge operation.

Test water during ballasting (source water prior to treatment, immediately after treatment) was stored about 24 hours until completion of discharge operation under the dark cold condition at a coastal test site on Imari bay, Saga Prefecture, Japan.

6.2 Brackish water requirement: 1st run (TR 23)

This test was performed to develop and transport test water for a test to confirm by-product generation under the brackish water requirement.

(1) Water quality

Table 6.2-1 shows results of water quality (water temperature, pH, salinity, dissolved oxygen, turbidity, TSS, POC and DOC).

The land-based test standard specifies salinity, TSS, DOC, and POC for the source water prior to treatment and untreated/control water during ballasting.

The definition of the brackish water requirement is 3~22 psu for salinity, more than 50 mg/L for TSS, more than 5 mg/L for DOC and more than 5 mg/L for POC.

This test was performed with conditions to satisfy these standards of water quality.

Table 6.2.1 Result of measurement and analysis of water quality
(brackish water requirement: 1st run (TR 23))

Process	sampling	Temp (°C)	pH value	Salinity (PSU)	DO (mg/L)	Turbidity (NTU)	TSS (mg/L)	POC (mg/L)	DOC (mg/L)
in take: before treatment	beginning	21.0	7.6	19.8	9.2	20.2	58	7.4	6.7
	middle	21.1	7.5	19.9	9.2	21.6	59	8.4	7.8
	end	21.6	7.5	19.9	9.3	20.6	51	7.8	7.2
intake: immediately after treatment	beginning	22.1	6.6	19.9	11.5	20.4	58	6.7	29.6
	middle	21.8	6.6	19.9	11.1	23.3	64	6.7	30.4
	end	22.2	6.6	19.9	10.4	24.3	60	5.7	30.8
discharge: before neutralization	beginning	20.8	6.4	19.9	13.2	25.8	39	3.7	28.8
	middle	21.0	6.4	19.9	13.0	23.0	43	4.2	28.8
	end	20.8	6.4	19.9	13.3	23.9	39	4.4	28.4
discharge: after neutralization	beginning	20.6	6.7	20.0	1.4	26.5	43	2.1	27.3
	middle	20.9	6.7	20.0	1.0	24.3	43	2.3	27.1
	end	21.0	6.6	19.7	0.9	19.6	37	2.3	25.9

(2) Others (items to be recorded during testing)

The following items were recorded when developing test water:

- .1 Flow amount on ballast line;
- .2 Dosing amount and concentration of PERACLEAN® Ocean when treating during ballasting;
- .3 Concentration of active substances (PAA and H₂O₂) by the PO concentration monitoring unit-1 and unit-2 on discharging treated water;
- .4 Dosing amount and concentration of sodium sulfite during treated water

- discharge and concentration of PAA and H_2O_2 by the PO concentration monitoring uni-2 after injection (control of neutralization treatment);
- .5 Flow amount to the sampling equipment when sampling; and
 - .6 Whether or not a dysfunction of system occurs and if so details of accident.

Figures 6.2-1~3 show the log data of these recording items of this test.

Flow amount steadily showed a little over 200 m^3/h . Dosing amount and concentration of PERACLEAN® Ocean during ballasting were 27 L/h as specified (in terms of ballast water volume of 207 m^3/h) and 150 mg/L respectively without much fluctuation.

Concentration of PAA and H_2O_2 of the treated water discharge was about 0.6 mg/L and 8.1 mg/L respectively and injection of sodium sulfite as neutralizer lowered these values to under detectable limit. 100 L/min. specified as value for dividing with “isokinetic flow speed was applied to sampling flow amount.

No particular troubles on recording neither dysfunction have been observed. Therefore, test system has been controlled as planned.

(3) Development and transportation of test water for a test to confirm by-product generation

Test water for a test to confirm by-product generation (source water prior to treatment, immediately after treatment and discharge water after neutralization) was transported under the dark cold condition to Idea Consultants, Inc., Yaizu City, Shizuoka, Japan in 10 hours after completion of discharge. Test water during ballasting (source water prior to treatment and immediately after treatment) was stored under the dark cold condition for 24 hours until completion of discharge at a coastal test site on Imari bay, Saga Prefecture, Japan.

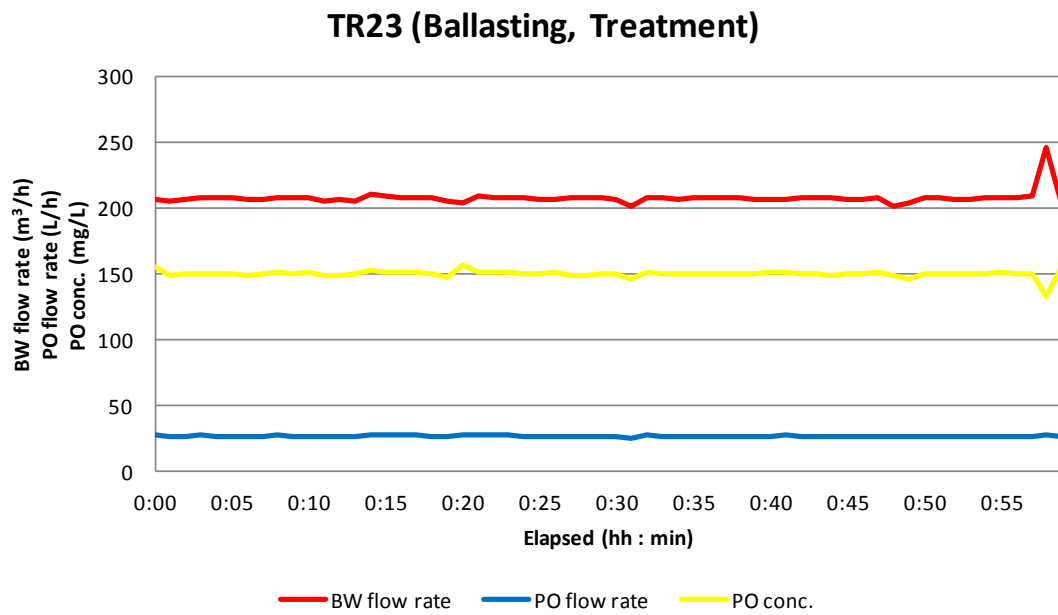


Figure 6.2-1 Control of treated ballasting water (1)

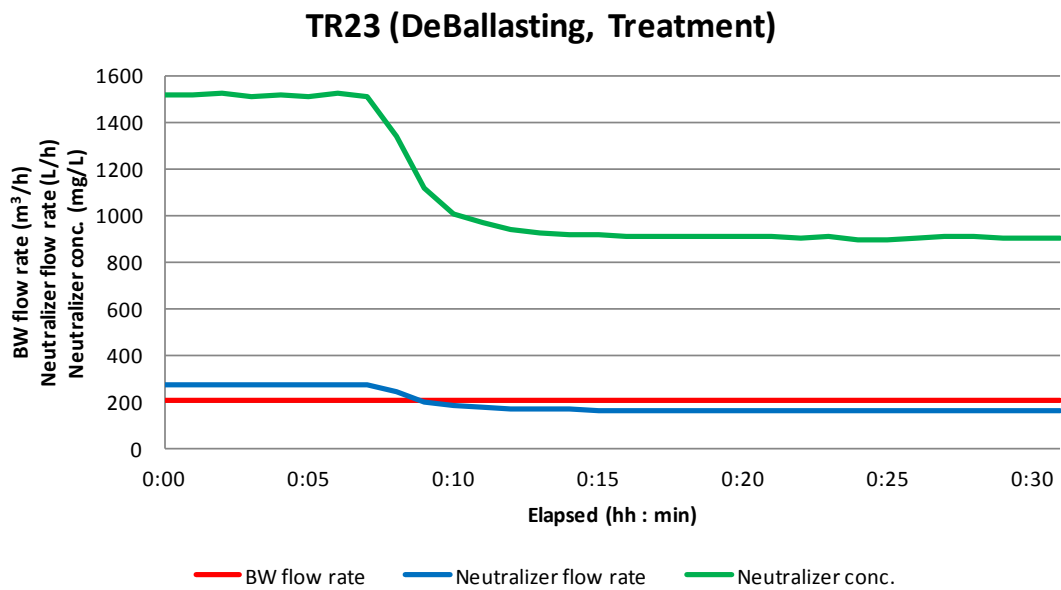


Figure 6.2-2 Control of treated discharge water (1)

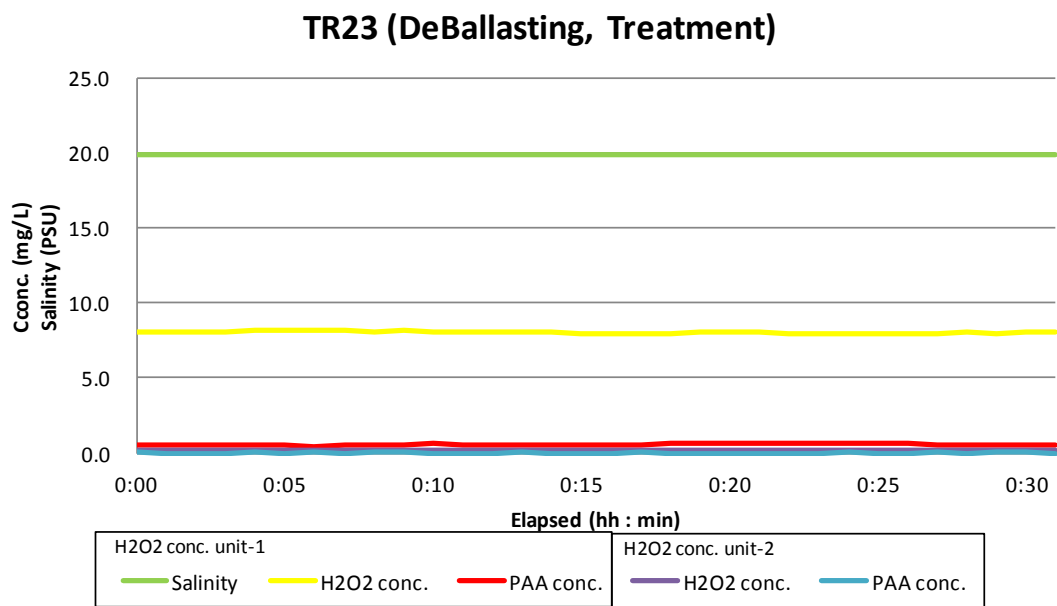


Figure 6.2-3 Control of treated discharge water (2)

6.3 Brackish water requirement: 2nd run (TR 31)

This test was conducted to evaluate compliance with D-2 standard under the brackish water requirement and also to develop and transport test water for a test to confirm by-product generation.

(1) Evaluation of compliance with D-2 standard

1) L size group

Table 6.3-1 shows the test result of L size group.

Since source water prior to treatment and untreated/control water during ballasting contained more than 10^5 inds./m³ and untreated/control discharge water contained more than 10^2 inds./m³, requirements of test were met. Treated water discharge contained less than 10 inds./m³ and complied with D-2 standard.

Table 6.3-1 Test result of L size group under brackish water requirement (2nd run (TR 31))
(unit: individual number (inds./m³))

		The total number of L size	The number of taxonomic groups	The number of species
Control	Beginning	2.9 $\times 10^5$	3	8
	Middle	3.1 $\times 10^5$	4	5
	End	3.9 $\times 10^5$	4	7
Before treatment	Beginning	3.5 $\times 10^5$	4	5
	Middle	3.8 $\times 10^5$	3	7
	End	3.8 $\times 10^5$	3	6
Immediately after treatment	Beginning	N.D.	N.D.	N.D.
	Middle	N.D.	N.D.	N.D.
	End	N.D.	N.D.	N.D.
One day after control	Beginning	51.6 $\times 10^2$	4	14
	Middle	79.0 $\times 10^2$	3	7
	End	59.2 $\times 10^2$	5	12
One day after treatment	Beginning	2 $\times 10^0$	2	2
	Middle	2 $\times 10^0$	1	1
	End	1 $\times 10^0$	1	1

N.D. indicates that organisms were not detected.

2) S size group

Table 6.3-2 shows test results of S size group.

Since source water and untreated/control water during ballasting contained more than 10^3 ind./ml and discharge of untreated/control water contained less than 10^2 /ml, requirements of test water met. Treated water discharge contained less than 10 ind./ml and complied with D-2 standard.

Table 6.3-2 Test result of S size group under brackish water requirement (2nd run (TR 31))
(unit: individual number (inds./ml))

		The total number of S size	The number of taxonomic groups	The number of species
Control	Beginning	2.59 $\times 10^3$	4	6
	Middle	2.39 $\times 10^3$	4	7
	End	2.33 $\times 10^3$	4	5
Before treatment	Beginning	1.59 $\times 10^3$	4	6
	Middle	2.27 $\times 10^3$	4	10
	End	2.65 $\times 10^3$	4	6
Immediately after treatment	Beginning	N.D.	N.D.	N.D.
	Middle	N.D.	N.D.	N.D.
	End	N.D.	N.D.	N.D.
One day after control	Beginning	12.60 $\times 10^2$	4	4
	Middle	14.56 $\times 10^2$	4	4
	End	20.40 $\times 10^3$	4	5
One day after treatment	Beginning	N.D.	N.D.	N.D.
	Middle	N.D.	N.D.	N.D.
	End	N.D.	N.D.	N.D.

N.D. indicates that organisms were not detected.

3) Bacteria

Table 6.3-3 shows test result of bacteria.

The land-based test standard specifies that the test should be implemented using test water with more than 10^4 cfu/ml of heterotrophic bacteria. This test complied with this standard.

(1) *Escherichia coli*

Colony forming unit number of *Escherichia coli* in the treated water discharge (after 1 day storage) was lower than the standard of ballast water discharge (lower than 250 cfu/100ml).

(2) Enterococci

Colony forming unit number of Enterococci in the treated water discharge (after 1 day storage) was lower than the standard of ballast water discharge (lower than 100 cfu/100 ml).

(3) Toxicogenic *Vibrio cholerae*

Colony forming unit number of toxicogenic *Vibrio cholerae* (*Vibrio cholera* O-1 and O-139) in the treated water discharge (after 1 day storage) was lower than the standard of ballast water discharge (lower than 1 cfu/100ml).

Table 6.3-3 Test result of bacteria under the brackish water requirement (2nd run (TR 31))

	Control						Treatment								
	Ballasting			One day after			Before			Immediately			One day after		
	Beginning	Midterm	End	Beginning	Midterm	End	Beginning	Midterm	End	Beginning	Midterm	End	Beginning	Midterm	End
<i>Vibrio cholerae</i> (First-stage)	3,900	6,200	1,080	2,500	2,480	2,500	4,460	940	1,680	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Vibrio cholerae</i> (Second-stage)	39	124	N.D.	156	N.D.	48	312	77	47	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Toxicogenic <i>Vibrio cholerae</i> (O1,O139)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Coliform	1,880	1,560	1,160	2,120	1,280	1,400	920	1,040	660	N.D.	N.D.	N.D.	2.6	0.8	0.2
<i>Escherichia coli</i>	180	120	160	180	140	120	260	140	100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Enterococcus group	5.0	7.8	7.4	1.8	3.0	3.2	5.2	3.4	4.8	N.D.	N.D.	N.D.	N.D.	0.2	N.D.
Enterococci	0.50	1.1	0.84	0.23	N.D.	0.091	1.3	0.85	0.81	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Heterotrophic bacteria	36,400	33,000	30,400	150,400	165,800	132,200	55,800	48,400	42,800	4.0	N.D.	10	148	124	130

Unit: Toxicogenic *Vibrio cholerae*, *Escherichia coli* and Enterococci (cfu/100ml), Heterotrophic bacteria (cfu/ml)

N.D. means that aforementioned bacteria were not detected.

4) Water quality

Table 6.3-4 shows the result of water quality (water temperature, pH, salinity, dissolved oxygen, turbidity, TSS, POC and DOC).

The land-based test standard specifies salinity, TSS, DOC and POC in the source water prior to treatment during ballasting and also untreated/control water.

For the brackish water requirement, 3~22psu for salinity, more than 50 mg/L for TSS. More than 5 mg/L for DOC and more than 5 mg/L for POC are defined.

This test has achieved these water quality standards.

Table 6.3-4 (1) Measurement and analysis result of water quality under the brackish water requirement (2nd run, treated water (TR 22))

Process	sampling	Temp (°C)	pH value	Salinity (PSU)	DO (mg/L)	Turbidity (NTU)	TSS (mg/L)	POC (mg/L)	DOC (mg/L)
in take: before treatment	beginning	10.3	7.9	20.7	9.2	53.4	72	6.7	5.6
	middle	10.4	7.9	20.6	9.0	41.6	80	7.0	6.1
	end	10.5	7.9	20.6	9.1	45.3	74	6.7	6.0
intake: immediately after treatment	beginning	10.4	6.6	20.8	9.5	48.7	79	6.5	27.9
	middle	10.5	6.6	20.7	9.2	42.9	80	6.4	29.8
	end	10.5	6.6	20.6	9.0	40.4	75	6.6	28.2
discharge: before neutralization	beginning	9.4	6.7	20.5	11.6	24.4	85	6.2	26.3
	middle	9.8	6.7	20.5	12.7	23.7	90	6.4	25.7
	end	9.7	6.7	20.6	12.4	26.4	81	6.2	28.4
discharge: after neutralization	beginning	9.8	6.8	20.6	1.0	38.5	62	2.2	21.9
	middle	10.0	6.7	20.6	0.7	33.3	52	2.8	24.0
	end	10.0	6.7	20.7	0.7	27.3	61	2.7	23.9

Table 6.3-4 (2) Measurement and analysis result of water quality under the brackish water requirement (2nd run (TR 22), untreated/control)

Process	sampling	Temp (°C)	pH value	Salinity (PSU)	DO (mg/L)	Turbidity (NTU)	TSS (mg/L)	POC (mg/L)	DOC (mg/L)
in take: control	beginning	10.4	7.7	20.3	8.3	65.2	79	7.4	6.1
	middle	10.8	7.7	20.2	8.5	61.4	83	6.9	6.5
	end	11.2	7.7	19.8	8.5	61.7	80	7.4	6.3
discharge: control	beginning	10.4	7.7	19.4	8.9	40.9	77	2.7	6.4
	middle	10.4	7.8	19.3	9.1	40.6	64	2.4	6.3
	end	10.5	7.8	19.4	9.0	40.0	71	2.4	6.4

5) Others (items to be recorded during tests)

The following items during tests have been recorded:

- .1 Flow amount on ballast line;
- .2 Dosing amount and concentration of PERACLEAN® Ocean in the treated water during ballasting;
- .3 Concentration of PAA and H₂O₂ in the treated water discharge by the PO concentration monitoring unit-1 and unit-2;
- .4 Dosing amount and concentration of sodium sulfite in the treated water discharge and concentration of PAA and H₂O₂ after injection by the PO concentration monitoring unit-2 (control of neutralization treatment);
- .5 Flow amount to the sampling equipment during sampling; and
- .6 Whether or not any dysfunction of system occurs and if so the detail.

Table 6.3-1~3 show the log data of recorded items of this test. Every flow amount was steadily a little over 200 m³/h. Dosing amount and concentration of PERACLEAN® Ocean in the treated water during ballasting were 27 L/h of the specified value (in terms of ballast water of 207 m³/h) and 150 mg/L respectively without much fluctuation.

Concentration of PAA and H₂O₂ in the treated water discharge prior to neutralization was about 1.5 mg/L and 9.6 mg/L and injection sodium sulfite as neutralizer lowered these values to under the detectable limit.

No particular error of recording neither dysfunction of operation have not been observed.

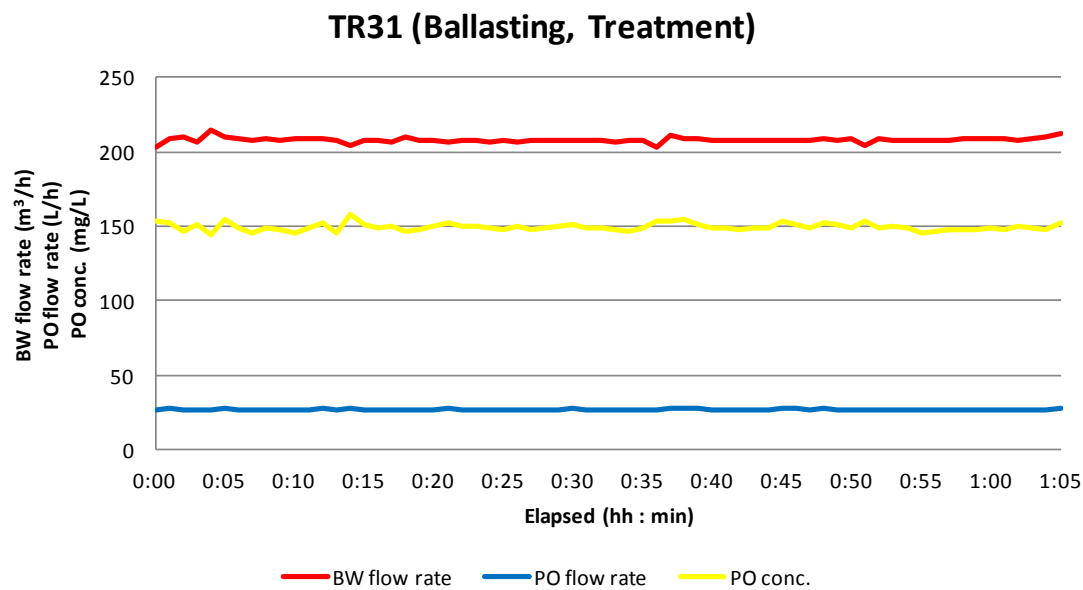


Figure 6.3-1 Control of treated water during ballasting

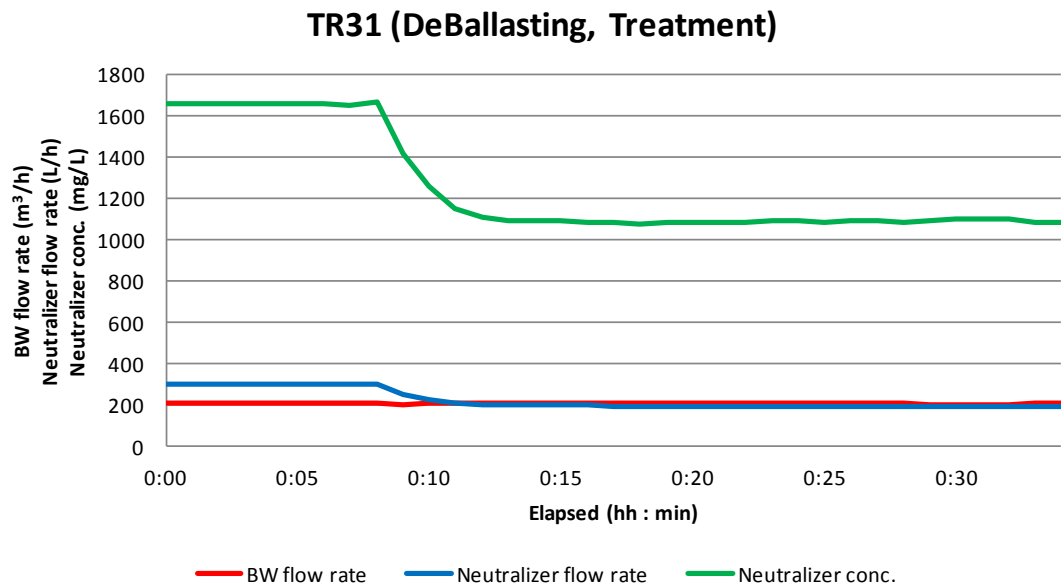


Figure 6.3-2 Control of treated water discharge (1)

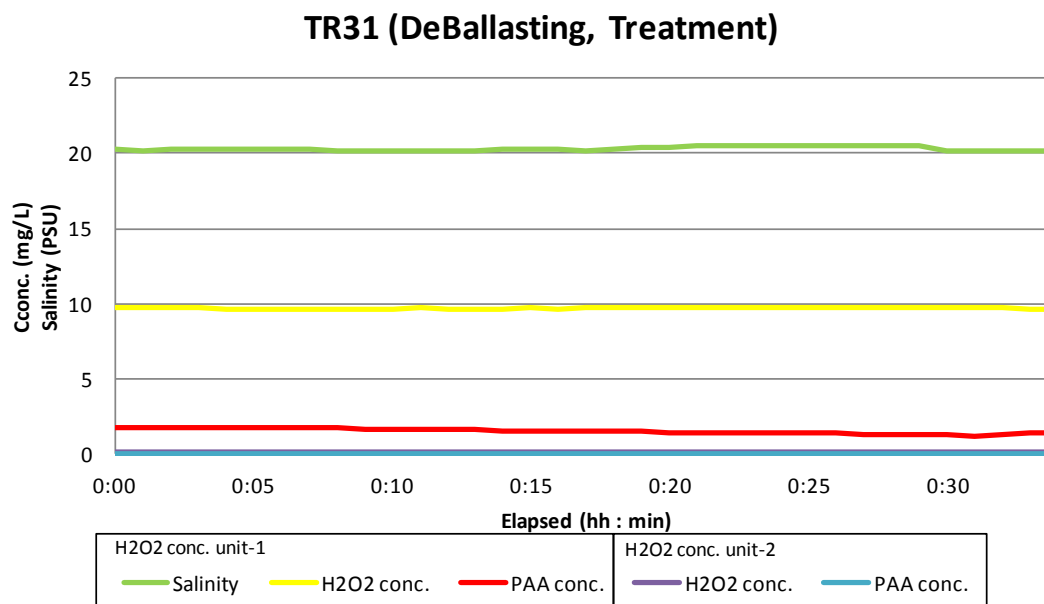


Figure 6.3-3 Control of treated water discharge (2)

(2) Development and transportation of test water for a test to confirm by-product generation

Test water for a test to confirm by-product generation (source water prior to treatment, immediately after treatment and discharge water after neutralization) was transported under the dark cold condition to Idea Consultants, Inc, Yaizu City, Shizuoka Prefecture, Japan in 10 hours after a completion of discharge operation. Test water during ballasting (source water prior to treatment and immediately after treatment) was stored under the dark cold condition for 24 hours until a completion of discharge operation at a coastal test site on Imari bay, Saga Prefecture, Japan.

6.4 Brackish water requirement: 3rd run (TR 35)

Development and transportation of test water for an aquatic toxicity test and a test to confirm by-product generation under the brackish water requirement were implemented during this test.

(1) Water quality

Table 6.4-1 shows the result of water quality (water temperature, pH, salinity, dissolved oxygen, turbidity, TSS, DOC and POC).

The land-based test standard specifies salinity, TSS, DOC, and POC for the source water prior to treatment during ballasting and for untreated/control water.

Under the brackish water requirement, it is required that salinity should be 3~22psu, TSS more than 50 mg/L, DOC more than 5 mg/L and POC more than 5 mg/L.

The test has been implemented under the condition in compliance with this water quality standard.

Table 6.4-1 Result of measurement and analysis of water quality
(brackish water requirement: 3rd run (TR 35))

Process	sampling	Temp (°C)	pH value	Salinity (PSU)	DO (mg/L)	Turbidity (NTU)	TSS (mg/L)	POC (mg/L)	DOC (mg/L)
in take: before treatment	beginning	11.7	7.8	22.1	8.9	53.4	120	10.5	6.7
	middle	11.8	7.9	22.1	9.0	52.9	100	9.3	6.9
	end	12.0	7.9	22.1	8.9	51.8	120	10.0	6.8
intake: immediately after treatment	beginning	11.4	6.3	21.9	9.5	36.0	120	8.9	29.0
	middle	11.3	6.2	22.1	9.6	33.3	110	9.0	32.8
	end	11.5	6.1	22.1	9.3	34.1	115	9.4	31.4
discharge: before neutralization	beginning	12.6	5.9	22.2	12.6	20.2	86	3.9	28.6
	middle	12.3	5.9	22.2	9.9	17.4	85	3.3	30.6
	end	12.0	6.0	22.1	10.6	17.4	89	4.0	31.7
discharge: after neutralization	beginning	13.1	6.1	22.3	0.6	16.6	61	3.4	23.4
	middle	12.7	6.1	22.3	0.6	16.7	64	2.4	24.7
	end	12.6	6.0	22.3	0.8	17.0	62	7.9	25.0

(2) Others (items to be recorded during tests)

The following items have been recorded when developing test water:

- .1 flow amount on ballast line;
- .2 Dosing amount and concentration of PERACLEAN® Ocean for treatment during ballasting;
- .3 Concentration of active substances (PAA and H₂O₂) in the treated water

- discharge by PO concentration monitoring unit-1 and unit-2;
- .4 Dosing amount and concentration of sodium sulfite as neutralizer when discharging treated water and concentration of PAA and H₂O₂ after injection by the PO concentration monitoring unit-2 (control of neutralization treatment);
 - .5 Flow amount to the sampling equipment when sampling; and
 - .6 Whether or not any dysfunction of system occurs and if so its detail.

Figures 6.4-1~3 show the log data of these recorded items of this test.

Flow amount was stable with a little over 200 m³/h. Dosing amount and concentration of PERACLEAN® Ocean when treating during ballasting were 27 L/h as specified (in terms of ballast water of 207 m³/h) 150 mg/L without much fluctuation.

Concentration of PAA and H₂O₂ in the treated water discharge prior to neutralization was about 0.4 mg/L and 9.4 mg/L respectively and injection of sodium sulfite of neutralizer lowered these values to under the detection limit.

Any error of recording and dysfunction of system have not been observed. This test system has been controlled as planned.

(3) Development and transportation of test water for an aquatic toxicity test and a test to confirm by-product generation

Test water for an aquatic toxicity test (source water prior to treatment and discharge water after neutralization) was transported under the dark cold condition to Chemicals Evaluation and Research Institute (CERI), Kurume City, Fukuoka Prefecture, Japan within 10 hours after completion of discharge operation. Test water for an aquatic toxicity test and a test to confirm by-product generation (source water prior to treatment, immediately after treatment, discharge water after neutralization) was transported under the dark cold condition to Idea Consultants, Inc., Yaizu City, Sizuoka Prefecture, Japan in 10 hours after completion of discharge. Test water during ballasting (source water prior to treatment and immediately after treatment) was stored for 24 hours until completion of discharge operation under the dark cold condition at a coastal test site on Imari bay, Saga Prefecture, Japan.

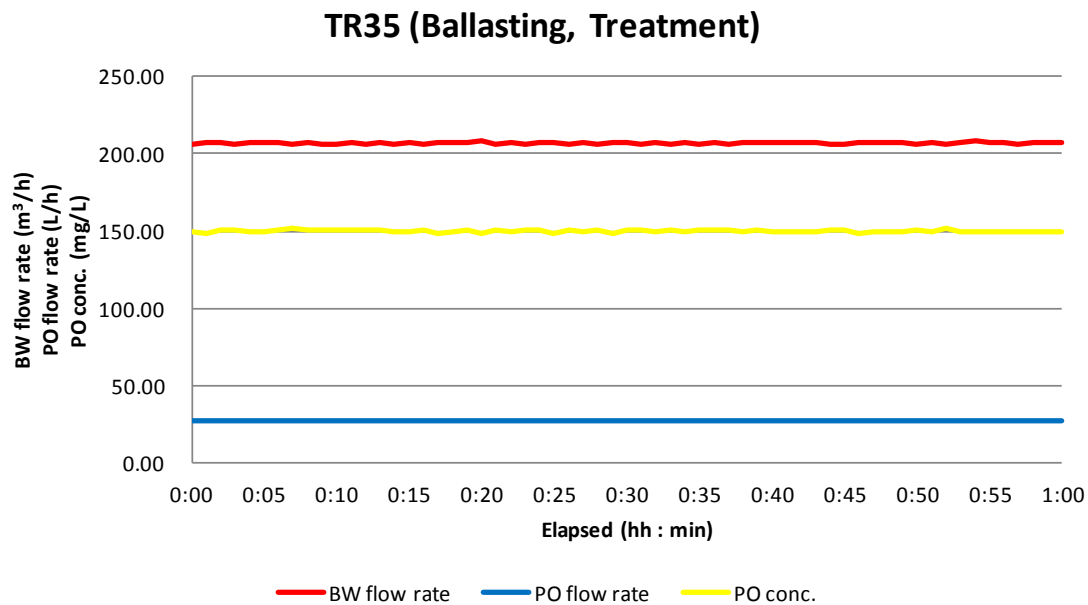


Figure 6.4-1 Control of treated water during ballasting

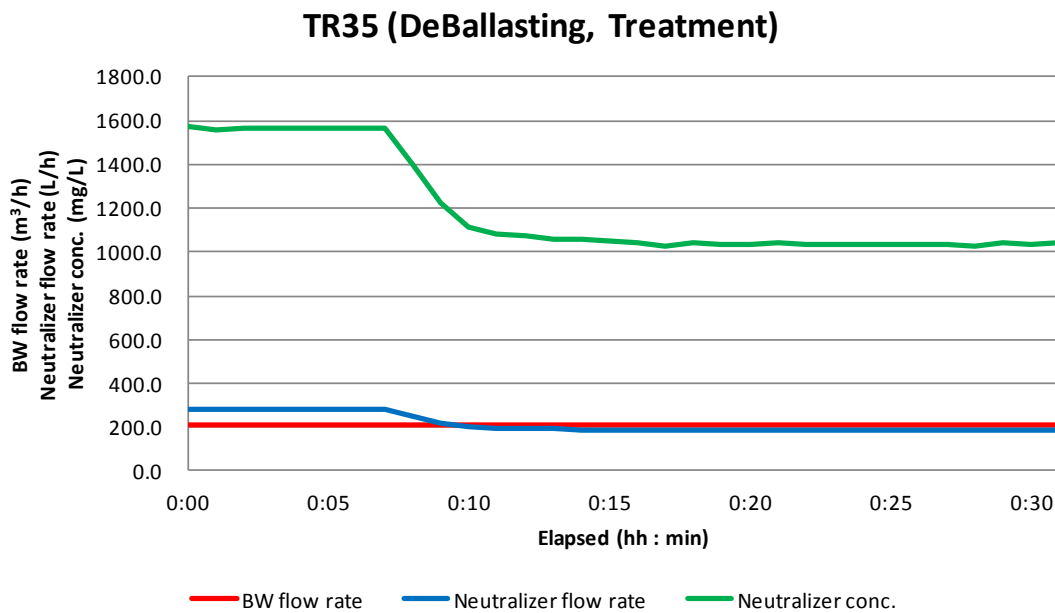


Figure 6.4-2 Control of treated water during discharge (1)

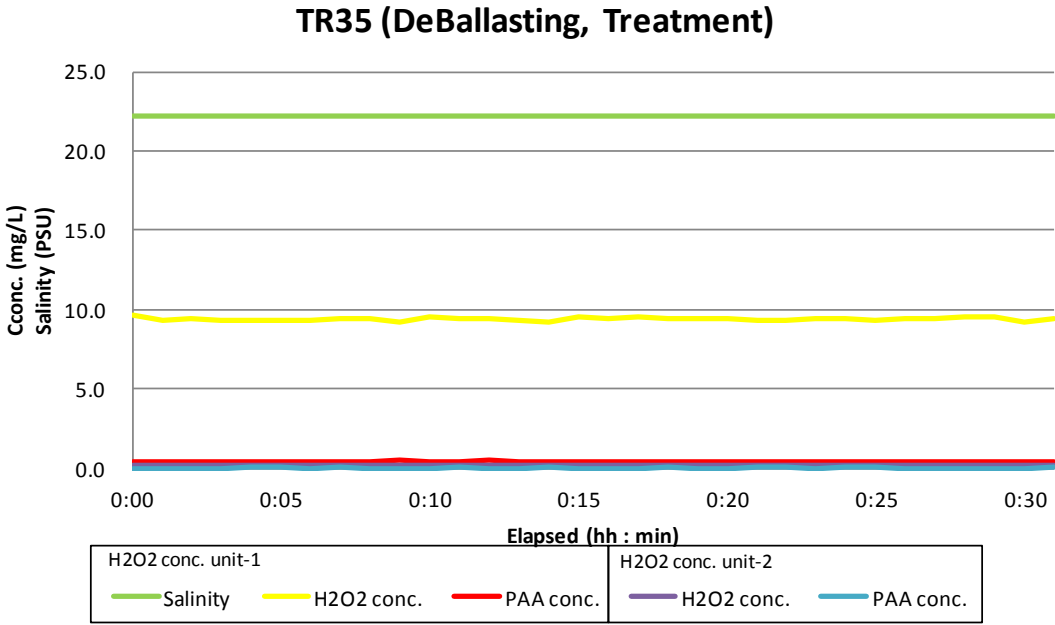


Figure 6.4-3 Control of treated water during discharge (2)